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ORIGINAL ARTICLE

Genotoxic Potential of Fluoxymesterone in Cultured Human Lymphocytes

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ABSTRACT

Fluoxymesterone, a synthetic androgenic anabolic steroid is used to treat breast cancer. Androgenic steroids have been reported to induce different types of cancer. In the present study we have tested the genotoxic potential of fluoxymesterone in cultured human peripheral blood lymphocytes. The study was performed in the absence as well as presence of metabolic activation with and without NADP, to see the effect of fluoxymesterone on chromosomal aberrations (CAs), mitotic index (MI), sister chromatid exchanges (SCEs) and replication index (RI). Fluoxymesterone neither induced CAs and SCEs nor affected MI and RI, significantly in the absence as well as in the presence of metabolic activation (S9 mix) without NADP. However, in the presence of metabolic activation with NADP it was found to be genotoxic at 30 and 40 µM.

Keywords: Anabolicsteroid, fluoxymesterone, genotoxicity

Introduction

Fluoxymesterone, a synthetic androgenic anabolic steroid is used to treat breast cancer[16,8,32]. It has similar properties like testosterone. The anti-tumour activity of fluoxymesterone appears related to reduction or competitive inhibition of prolactin receptors or estrogen receptors[21,5]. The use of synthetic androgenic steroids has been reported to induce different types of cancer[33,23,35]. Fluoxymesterone was reported to induce chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in cultured human peripheral blood lymphocytes and also increase frequencies of micronuclei and SCEs in bone marrow cells of mice. However, in Ames salmonella assay it was found negative in both with and without S9 mix[4]. In the present study we have studied a wide range of different doses of fluoxymesterone in the absence as well as presence of metabolic activation (with and without NADP) to see the effect on chromosomal aberrations (CAs), mitotic index (MI), sister chromatid exchanges (SCEs) and replication index (RI) in human lymphocytes *in vitro*.

Material and method

Chemicals

Fluoxymesterone (CAS: 76-43-7), hoechst 33258, mitomycin C and cyclophosphamide were procured from Sigma. RPMI 1640, phytohaemagglutinin-M and antibiotic-antimycotic mixture were procured from Gibco. Dimethyl sulphoxide and giemsa stain were procured from E. Merk. Colchicine was procured from Micro lab and 5-bromo-2-deoxyuridine was procured from SRL, India.

Human lymphocyte culture

Duplicate peripheral blood cultures were treated according to Carballo *et al*[2]. Briefly, 0.5 ml of heparinized blood sample was obtained from a healthy female donor and was placed in a sterile

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culture bottle containing, 7 ml of RPMI 1640, supplemented with 1.5 ml of fetal calf serum, 0.1 ml of antibiotic-antimycotic mixture and 0.1 ml of phytohaemagglutinin. The culture bottles were placed in an incubator at 37°C for twenty-four hour. Untreated, negative and positive controls were also performed simultaneously.

Chromosomal aberrations analysis

After twenty-four hour, fluoxymesterone at 1, 5, 10, 20, 30 and 40 mM, dissolved in dimethylsulphoxide (DMSO) was added. The cells were cultured for another forty-eight hour at 37°C in the incubator. For metabolic activation experiments, 0.5 ml of S9 mix (rat liver) was given along with each of the tested doses of fluoxymesterone. S9 mix was prepared from the liver of healthy rats (Wistar Strain) as per standard procedures of Maron and Ames[18]. The S9 fraction was enhanced by addition of 5 mM of NADP and 10 mM of glucose-6-phosphate just before use. The S9 mix without NADP was also given with each of the tested dose of fluoxymesterone. After three hour the S9 mix was removed by washing the cells. An amount of 0.2 ml of colchicine (0.2 mg/ml) was added to the culture bottle one hour prior to harvesting. Cells were centrifuged at 1000 rpm for ten minutes. The supernatant was removed and 5 ml of prewarmed (37°C) 0.075 molar KCl (hypotonic solution) was added. Cells were resuspended and incubated at 37°C for fifteen minutes. The supernatant was removed by centrifugation and 5 ml of chilled fixative (methanol: glacial acetic acid; 3:1) was added. The fixative was removed by centrifugation and the procedure was repeated twice. To prepare slides, 3-5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for fifteen minutes. Three hundred metaphases were examined for the occurrence of different types of abnormalities. Criteria to classify different types of aberrations were followed in accordance with the recommendation of Environmental monitoring of human populations[13]. The mitotic index (MI) was scored as the number of metaphases among 1000 lymphocytes nuclei and expressed as a percentage.

Sister chromatid exchange analysis

For sister chromatid exchange analysis, bromodeoxyuridine (BrdU, 10 mg/ml) was added at the beginning of the culture. After twenty-four hour 1, 5, 10, 20, 30 and 40 mM of fluoxymesterone, dissolved in DMSO, was added and kept for another forty-eight hour at 37°C in an incubator. For metabolic activation experiments, 0.5 ml of S9 mix with and without NADP was given along with each of the tested dose. After three hour the S9mix was removed by washing. Mitotic arrest was done one hour prior to harvesting by adding, 0.2 ml of colchicine (0.2 mg/ml). Hypotonic treatment and fixation were performed in the same way as described for CAs analysis. The slides were processed according to Perry and Wolff[22]. The sister chromatid exchange induction was analysed from the fifty second division mitoses per dose.

Cell cycle kinetics

Cells undergoing, first (M_1), second (M_2) and third (M_3) metaphase divisions were detected with BrdU-Harlequin technique for differential staining of metaphase chromosomes[34,23], both in the absence and presence of metabolic activation (S9 mix), with and without NADP. Treatments were similar as described earlier in the text. Two hundred cells were scored per treatment for the analysis of cell cycle kinetics. The replication index (RI) an indirect measure of studying cell cycle progression was calculated by applying the following formula[14].

$$R1 = \frac{M_1 + 2M_2 + 3M_3}{100}$$

Statistical analysis

Student's *t*-test was used for the analysis of CAs and SCEs, whereas Chi-square test (c^2) was used to analyse the cell cycle kinetics. The level of significance was tested from standard statistical tables of Fisher and Yates[7] (1963).

Results and discussions

Results

Fluoxymesterone neither induced chromosomal aberrations (Table 1 and 2) and sister chromatid exchanges (Table 4) nor affected mitotic index (Table 1 and 2) and replication index (Table 4) at significant level even at the highest tested dose i.e. 40 mM, in the absence as well as presence of metabolic activation (S9 mix) without NADP. However, it was found to be genotoxic at 30 and 40 mM in the presence of metabolic activation with NADP. A significant increase in the chromosomal aberrations (Table 3) and sister chromatid exchanges (Table 4) was observed at 30 and 40mM of fluoxymesterone in the presence of metabolic activation with NADP. A significant decrease in the mitotic index (Table 3) and replication indices (Table 4) was observed at 30 and 40mM of fluoxymesterone in the presen

Table 1: Chromosomal aberrations and mitotic index in human lymphocytes treated with fluoxymesterone in the absence of S9 mix.

Treatment	Cells scored	Abnormal metaphases without gaps		Chromosomal aberrations					MI %
		Number	Mean%±SE	Gaps	CTB	CSB	CTE	DIC	
			Fluoxymesterone (mM)						
1	300	2	0.6±0.4	2	2	-	-	-	2.6±0.5
5	300	4	1.3±0.6	2	2	2	-	-	2.5±0.4
10	300	6	2.0±0.8	3	4	2	-	-	2.5±0.4
20	300	6	2.0±0.8	3	3	3	-	-	2.3±0.4
30	300	4	2.3±0.8	4	4	3	-	-	2.3±0.4
40	300	7	2.3±0.8	3	4	3	-	-	2.2±0.3
Untreated	300	2	0.6±0.4	1	2	-	-	-	2.8±0.5
Negative control (DMSO, 5 ml/ml)	300	2	0.6±0.4	1	2	-	-	-	2.6±0.5
Positive control (Mitomycin C, 0.3 mg/ml)	300	27	9.0±1.6[a]	15	19	10	4	2	0.8±0.2[a]

CTB: Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric; SE: Standard error.
[a]Significant difference with respect to untreated (t-test, P<0.05).

Table 2: Chromosomal aberrations and mitotic index in human lymphocytes treated with fluoxymesterone in the presence of S9 mix.

Treatment	Cells scored	Abnormal metaphases without gaps		Chromosomal aberrations					MI %
		Number	Mean%±SE	Gaps	CTB	CSB	CTE	DIC	
			Fluoxymesterone (mM)						
1	300	3	1.0±0.5	2	2	1	-	-	2.5±0.4
5	300	3	1.0±0.5	2	2	1	-	-	2.4±0.4
10	300	6	2.0±0.8	3	3	3	-	-	2.3±0.4
20	300	7	2.3±0.8	3	4	3	-	-	2.3±0.4
30	300	7	2.3±0.8	4	4	3	-	-	2.2±0.3
40	300	8	2.6±0.9	4	5	3	-	-	2.1±0.3
Untreated	300	2	0.6±0.4	1	2	-	-	-	2.9±0.5
Negative control (DMSO, 5 ml/ml)	300	3	1.0±0.5	2	2	1	-	-	2.7±0.5
Positive control (Cyclophosphamide, 0.16 mg/ml)	300	32	10.7±1.7[a]	19	24	15	5	4	0.6±0.2[a]

CTB: Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric; SE: Standard error.
[a]Significant difference with respect to untreated (t-test, P<0.05).

Table 3: Chromosomal aberrations and mitotic index in human lymphocytes treated with fluoxymesterone in the presence of S9 mix with NADP.

Treatment	Cells scored	Abnormal metaphases without gaps		Chromosomal aberrations					MI %
		Number	Mean%±SE	Gaps	CTB	CSB	CTE	DIC	
			Fluoxymesterone (mM)						
1	300	3	1.0±0.5	2	2	1	-	-	2.5±0.4
5	300	5	1.7±0.7	3	3	2	-	-	2.4±0.4
10	300	7	2.3±0.8	3	4	3	-	-	2.3±0.4
20	300	7	2.3±0.8	3	52	2	-	-	1.5±0.3[a]
30	300	10	3.3±1.0[a]	5	6	4	-	-	1.2±0.3[a]
40	300	14	4.6±1.2[a]	9	8	6	-	-	2.2±0.4
Untreated	300	2	0.6±0.4	1	2	-	-	-	2.7±0.5
Negative control (DMSO, 5 ml/ml)	300	2	0.6±0.4	1	2	-	-	-	2.7±0.5
Positive control (Cyclophosphamide, 0.16 mg/ml)	300	34	11.3±1.8[a]	18	21	16	7	5	0.7±0.2[a]

CTB: Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric; SE: Standard error.
[a]Significant difference with respect to untreated (t-test, P<0.05).

Discussion

The results of the present investigation reveal that fluoxymesterone is genotoxic only in the presence of metabolic activation (S9 mix) with NADP. The study performed by Dhillon *et al*[4], showed that fluoxymesterone induced CAs and SCE both in the presence as well as absence of S9 mix in cultured human peripheral blood lymphocytes. Earlier studies performed on the genotoxic potential of synthetic progestins and estrogens reveal that estrogens and few synthetic progestins are genotoxic both in the presence as well as absence of metabolic activation[12,19,31] but few synthetic progestins/ estrogens requires metabolic activation to act as a genotoxic agent[24,25,28,25]. On comparing the potential of synthetic progestins for the DNA adduct formation, it was found that cyproterone acetate,

Table 4: Sister chromatid exchanges and cell growth kinetics in human lymphocytes exposed to fluoxymesterone.

Group	SCEs/Cell (Mean±SE)	Range	RI
	Without S9 mix Fluoxymesterone (mM)		
1	1.92±0.28	0 – 5	1.79
5	2.33±0.37	0 – 5	1.77
10	2.75±0.50	0 – 5	1.76
20	2.96±0.56	0 – 5	1.75
30	3.02±0.61	1 – 5	1.74
40	3.04±0.64	1 – 5	1.72
Untreated	1.58±0.17	0 – 5	1.84
Negative control (DMSO, 5 ml/ml)	1.48±0.16	0 – 5	1.8
Positive control (Mitomycin C, 0.3 mg/ml)	11.43±0.84[a]	2 – 13	1.37[b]
	With S9 mix without NADP Fluoxymesterone (mM)		
1	2.01±0.31	0 – 5	1.79
5	2.70±0.48	0 – 5	1.76
10	2.72±0.52	1 – 5	1.75
20	2.76±0.54	1 – 5	1.75
30	2.94±0.55	1 – 5	1.74
40	2.98±0.58	1 – 5	1.7
Untreated	1.66±0.21	0 – 5	1.84
Negative control (DMSO, 5 ml/ml)	1.42±0.13	0 – 5	1.82
Positive control (Cyclophosphamide, 0.16 mg/ml)	12.57±0.93[a]	2 – 14	1.34[b]
	With S9 mix with NADP Fluoxymesterone (mM)		
1	2.11±0.34	0 – 5	1.78
5	2.85±0.53	0 – 5	1.76
10	3.21±0.69	1 – 5	1.74
20	3.33±0.71	1 – 5	1.72
30	6.66±0.82[a]	2 – 7	1.52[b]
40	7.34±0.88[a]	2 – 8	1.48[b]
Untreated	1.69±0.23	0 – 5	1.83
Negative control (DMSO, 5 ml/ml)	1.46±0.15	0 – 5	1.82
Positive control (Cyclophosphamide, 0.16 mg/ml)	12.24±0.91[a]	2 – 14	1.32[b]

Dimethylsulphoxide; S.E.: Standard error. RI: Replication index

[a]Significant difference with respect to untreated (t-test, $P<0.05$). [b]Significant difference with respect to untreated (Chi-square test, $p<0.05$)

megestrol acetate and chlormadinone acetate are potent enough to form adducts in human liver slices *in vitro*[6,36]. Looking at the structure of these synthetic progestins the double bond between carbon-6 and carbon-7 is present (Fig. 1). The bioactivation of cyproterone acetate involves the reduction of the keto group at carbon-3 followed by sulfonation of the hydroxy steroid[15,37]. The resulting sulfo conjugate is supposed to be very unstable and can decompose to a reactive DNA-binding carbonium ion. Estradiol-17b and ethinylestradiol failed to form DNA adducts in human liver slices *in vitro*, and the double bond between carbon-6 and carbon-7 in them is absent[6]. However, medroxyprogesterone acetate induced DNA adducts only in some human liver slices though the double bond between carbon-6 and carbon-7 is absent[6] and it requires metabolic activation to show the genotoxic effects[28]. Synthetic progestins like cyproterone acetate, chlormadinone acetate and megestrol acetate undergo nucleophilic reaction and thereby generate reactive oxygen species that are responsible for the genotoxic damage[26,27,30]. Hence, it can be concluded from earlier studies that progestins in which double bond between carbon-6 and carbon-7 is present they may undergo nucleophilic reaction and progestins in which double bond between carbon-6 and carbon-7 is absent they undergo metabolic activation in the presence of NADP to show some genotoxic action like estrogens[28] (Fig. 1). In estrogens such as estradiol-17b and ethinylestradiol double bond between carbon-6 and carbon-7 is absent. In our present study with fluoxymesterone, double bond between carbon-6 and carbon-7 is absent and it showed genotoxic effect only in the presence of metabolic activation with NADP. Cytochrome P450s in liver S9 fractions plays an important role in activating promutagens to proximate and/or ultimate mutagens. Rat and human liver P450s are involved in the activation of some chemical carcinogens having different isoforms[9] The carbonyl group has a certain degree of polar character, which increases with unsaturation so that it has more single bond character than that of a saturated ketone and is also more ionic in nature. Due to this property estrogens (estradiol-17b, ethinylestradiol) undergo metabolic activation by cytochrome P450. The first step may involve is the aromatic hydroxylation forming catechol metabolites[8,17] The redox cycling between various forms of quinones may give rise to the reactive oxygen species that are responsible for the DNA damage[8]. An increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes is associated with an increased overall risk of cancer[11,10]. The ready quantifiable nature of sister chromatid exchanges with high sensitivity for revealing toxicant-DNA interaction and the demonstrated ability of genotoxic chemicals to induce

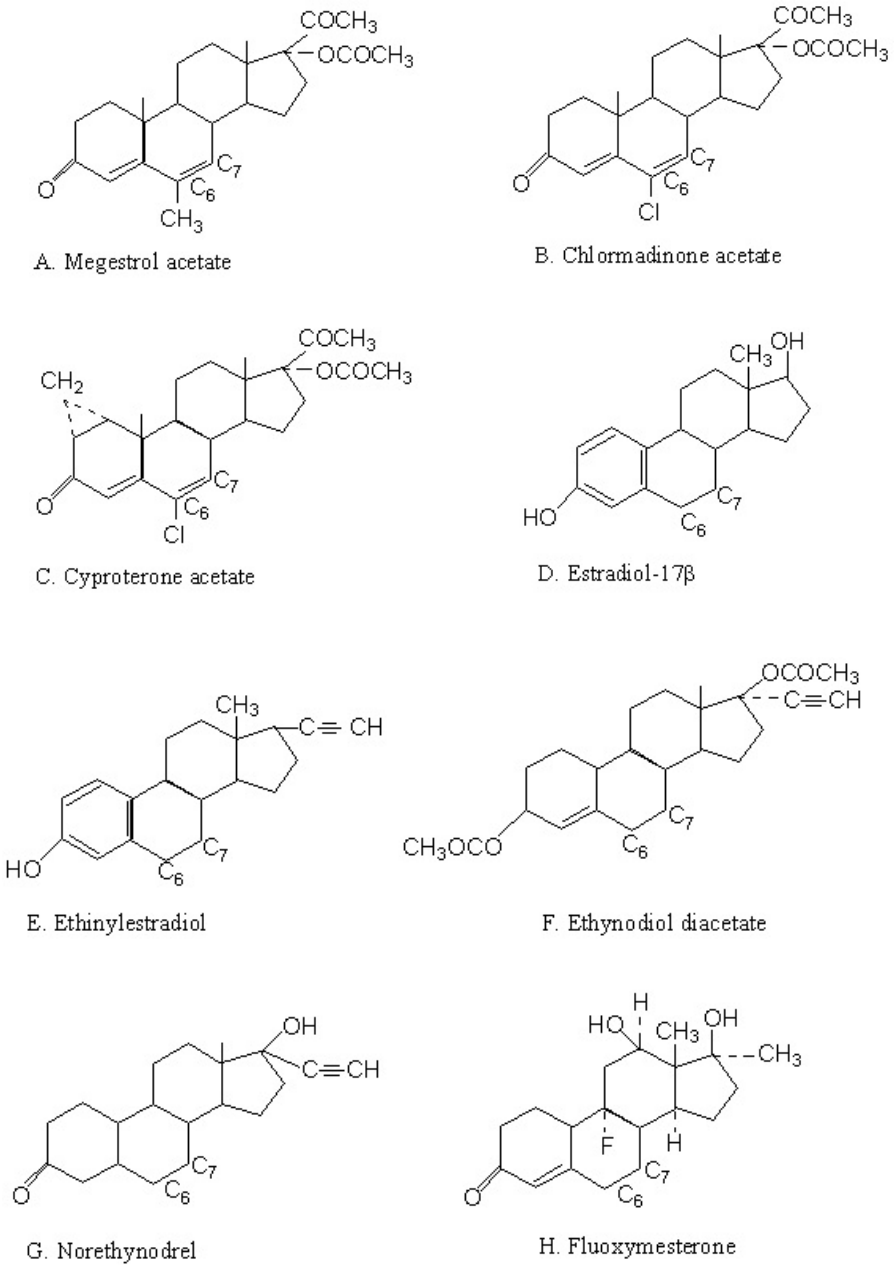

Fig. 1: Structure of some synthetic progestin and estrogen. Presences of double bond between carbon-6 (A-C), Absence of double bond between carbon-6 and carbon-7 (D-H).

significant increase in sister chromatid exchanges in cultured cells has resulted this endpoint being used as indicator of DNA damage in blood lymphocytes of individuals exposed to genotoxic carcinogens[1]. Synthetic steroids after oral administration readily absorbed through the intestinal lining with maximum plasma concentration being reached within 30-60 min, then they undergo first pass metabolism in liver[20]. Halostestin® tablet contains 2-10 mg of fluoxymesterone, but the blood plasma concentrations do not reach the highest tested dose (40 mM) under normal circumstances. However, markedly higher blood concentrations may be reached in some clinical conditions[20] hence the present study was performed on cultured human lymphocytes, using a wide range of concentrations of fluoxymesterone.

Conclusion

Fluoxymesterone is genotoxic only in the presence of metabolic activation (S9 mix) with NADP at 30 and 40mM.

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References

1. Albertini, R.J., D. Anderson, G.R. Douglas, L. Hagmar, K. Heminiki, F. Merelo, A.T. Natrajan, H. Norppa, D.E.G. Shuker, R. Tice, M.D. Walters and A. Aitio, 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutation Research, 463: 111-172.
2. Carballo, M.A., S. Alvarez and A. Boveris, 1993. Cellular stress by light and Rose Bengal in human lymphocytes. Mutation Research, 288: 215-222.
3. Crossen, P.E and W.F. Morgan, 1977. Analysis of human lymphocyte cell cycle time in culture measured by sister chromatid differentiated staining. Experimental Cell Research, 104: 453-457.
4. Dhillon, V.S, J. Singh, H. Singh and R.S. Kler, 1995. In vitro an in vivo genotoxicity of hormonal drugs VI. Fluoxymesterone. Mutation Research, 342: 103-111.
5. Dorr, R.T. and D.D. VonHoff, 1994. Cancer chemotherapy handbook, 2nd ed. Norwalk: Appleton & Lange, 32: 189-94.
6. Feser, W., R.S. Kerdar, A. Boumann, J. Korber, H. Blode and W. Kuhnz, 1998. DNA adduct formation of selected sex steroids in human liver slices in vitro. Toxicology in vitro, 12: 353-364.
7. Fisher, R.A and Y. Yates, 1963. Statistical Table for Biological Agricultural and Medical Research Workers. Sixth ed. Oliver and Boyd, Edinburgh, pp 138.
8. Fishman, J., 1983. Aromatic hydroxylations of estrogens. Annual Review of Physiology, 45: 61-72.
9. Fornasiero, A., E. Ferrazzi, S.M. Aversa, O. Daniele, C. Ghiotto, V.C. Sileni, P. De Besi and M.V. Fiorentino, 1992. Deflazacort and fluoximesterone in advanced, pretreated breast cancer. Tumorigenesis, 78: 266-9.
10. Guengerich, F.P. and T. Shimada, 1991. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chemical Research and Toxicology, 4: 391-407.
11. Hagmar, L., A. Brogger, I.L. Hansteen, S. Heims, B. Hogstedt, L. Knudsen, B. Lambert, K. Linnainmaa, F. Mitelman, I. Nordenson, C. Reuterwall, S. Salomaa, S. Skerfving and M. Sorsa, 1994. Cancer risk in humans predicted by increased level of chromosomal aberration in human lymphocytes: Nordic study Group on the Health Risk of chromosome Damage. Cancer Research, 54: 2919-2922.
12. Hagmar, L., S. Bonassi, U. Stromberg, A. Brogger, J.E. Knudson, H. Norppa and C. Reuterwall, 1998. Chromosomal aberrations in human lymphocytes predict human cancer: a report from thre European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Research, 58: 4117-4121.
13. Hundal, B.S., V.S. Dhillon and I.S Sidhu, 1997.Genotoxic potential of estrogens. Mutation Research, 389: 173-181.
14. IPCS., 1985. International Programme on Chemical Safety. Environmental Health Criteria 46, Guidelines for the study of Genetics Effects in Human Populations, Vol. 46, WHO, Geneva pp: 25-54.
15. Ivett, J.L. and R.R. Tice, 1982. Average generation time: a new method of analysis and quantitation of cellular proliferation kinetics. Environmental Mutagenesis, 4: 358.
16. Kerdar, R.S., A. Baumann, M. Brudny-Klopped, H. Biere, H. Blode and W. Kuhnz, 1995. Identification of 3a-hydroxy-cyproterone acetate as a metabolite of cyproterone acetate in the bile of female rats and the potential of this and other already known or putative metabolites to form DNA adducts in vitro. Carcinogenesis, 16: 1835-1841.
17. Loprinzi, C.L., J.W. Kugler, J.A. Sloan, J.A. Mailliard, J.E. Krook, M.B. Wilwerding, K.M. Rowland Jr, J.K. Camoriano, P.J. Novotny and B.J. Christensen, 1999. Randomized comparison of megestrol acetate versus dexamethasone versus fluoxymesterone for the treatment of cancer. Journal of Clinical Oncology, 17: 3299-306.
18. Maclusky, N.J., F. Naftolin, L.C. Krey and S. Franks, 1981. The catechol estrogens. Journal of Steroid Biochemistry, 15: 111-124.
19. Maron, D.M and B.N. Ames, 1983. Revised methods for the salmonella mutagenicity test. Mutation Research, 113: 173-215.
20. Martelli, A. and G. Brambilla, 2002. Are some progestins genotoxic liver carcinogens? Mutation Research, 512: 155-163.
21. Martelli, A., F. Mattioli, M. Angiola, R. Reimann and G. Brambilla, 2003. Species, sex and interindividual differences in RNA repair induced by nine sex steroids in primary cultures of rat and human hepatocytes. Mutation Research, 536: 69-78.
22. McEvoy, G.K., 1993. American hospital formulary service: Drug information 1993. American Society of Hospital Pharmacists, Bethesda, 1915-7.

23. Perry, P. and S. Wolff, 1974. New Giemsa method for the differential staining of sister chromatids. Nature, 251: 156-158.
24. Sale, G.E. and K.G. Lerner, 1977. Multiple tumors after androgen therapy. Archives of Pathology and Laboratory Medicine, 101: 600-603.
25. Siddique, Y.H. and M. Afzal, 2004a. Evaluation of genotoxic potential of synthetic progestin ethynodiol diacetate in human lymphocytes in vitro. Current Science, 86: 1161-1165.
26. Siddique, Y.H. and M. Afzal, 2005a. Evaluation of genotoxic potential of norethynodrel in human lymphocytes in vitro. Journal of Environmental Biology, 26: 387-392.
27. Siddique, Y.H. and M. Afzal, 2004b. Induction of chromosomal aberrations and sister chromatid exchanges by chlormadinone acetate: a possible role of reactive oxygen species. Indian Journal of Experimental Biology, 42: 1078-1083.
28. Siddique, Y.H. and M. Afzal, 2005b. Genotoxic potential of cyproterone acetate: a possible role of reactive oxygen species. Toxicology *in vitro*, 19: 63-68.
29. Siddique, Y.H., G. Ara, T. Beg and M. Afzal, 2006. Genotoxic potential of medroxyprogesterone acetate in cultured human peripheral blood lymphocytes. Life Sciences, 80: 212-218.
30. Siddique, Y.H., T. Beg and M. Afzal, 2005a. Genotoxic potential of ethinylestradiol in cultured mammalian cells. Chemico Biological Interactions, 151: 133-141.
31. Siddique, Y.H., T. Beg and M. Afzal, 2005b. Antigenotoxic effects of ascorbic acid against megestrol acetate induced genotoxicity in mice. Human and Experimental Toxicology, 24: 121-127.
32. Singh, H., J.R. Singh, V.S. Dhillon, D. Bali and H.P. Sharma, 1994. In vitro and *in vivo* genotoxicity evaluation of hormonal drugs.II. Dexamethasone. Mutation Research, 308: 89-97.
33. Skeel, R.T., J.W. Andersen, D.C. Tormey, A.B. Benson, R.F. Ashbury and G. Falkson, 1989. Combination chemotherapy of advanced breast cancer. Comparison of dibromodulcitol, doxorubicin, vincristine, and fluoxymesterone to thiotepa, doxorobicin, vinblastine and fluoxymesterone: an Eastern Cooperative Oncology Group Study. Cancer, 64: 1393-9.
34. Sweeney, E.C. and D.J. Evans, 1976. Hepatic lesions in patients treated with synthetic anabolic steroids. Journal of Clinical Pathology, 29: 626-633.
35. Tice, R.R., E.L. Schneider and J.M. Rary, 1976. The utilization of bromodeoxyuridine in corporation into DNA for the analysis of cellular kinetics. Experimental Cell Research, 102: 232-236.
36. Treuner, J., D. Niethammer, A. Flach, H. Fischbach and K.W. Schenc, 1980. Hepatocellular carcinoma following oxymetholone treatment (Ger.) Med Welt, 31: 952-955.
37. Werner, S., S. Kunz, T. Beckurts, C.D. Heidecke, T. Wolff and L.R. Schwarz, 1997. Formation of DNA adducts by cyproterone acetate and some structural analogues in primary cultures of human hepatocytes. Mutation Research, 395: 179-187.
38. Werner, S., S. Kunz, T. Wolff and L.L. Schwarz, 1996. The steroidal drug cyproterone acetate is activated to DNA binding metabolites by sulfonation. Cancer Research, 56: 4391-4397.